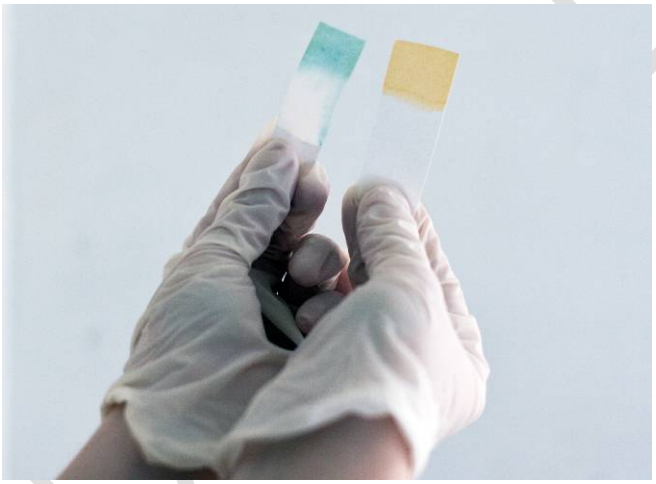
# PROTEIN TEST:
# VALIDATION OF REENGINEERED REAGENT





## Report Draft, June 2011

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Important Note: This report is a preliminary report for a four-part study. Two parts remain to be completed. The results herein are only meant for reporting purposes to the donor. Distribution of these results beyond this purpose may jeopardize chances of publication in peer-reviewed journals which is planned after the full study is completed.

# Summary

Pre-eclampsia/eclampsia is one of the leading causes of maternal deaths in the world—contributing between 8–25% of maternal mortality globally. In Nepal PE/E is the cause of 21% of maternal deaths, now the second leading cause of maternal deaths after PPH deaths due to PE/E. It can be prevented through early detection of elevated blood pressure and proteinuria usually during antenatal care (ANC).

Worldwide, there is a significant unmet need for protein testing. In Nepal 30% of pregnant women attend ANC fourth visit, but many do not get the protein test due to cost or unavailability. There are several conventional diagnostic tests for proteinuria available in the market, dipstick being the leading method used. These tests are either dependent on laboratory infrastructure, designed for use by only trained health providers, or are expensive for most low-resource settings. Jhpiego in collaboration with Johns Hopkins University School of Biomedical Engineering Center for Bioengineering Innovation and Design (JHU-CBID) has developed a simple protein test that is lower cost, easy to interpret, and designed for self-test. The new protein test consists of a filter paper strip which is marked at one end with modified reagent dispensed from a felt tip marker pen. Preliminary tests in the design laboratory at JHU reengineered the reagent to yield dichotomous result, and developed the dispensing platform.

The test is being evaluated in a four-step process in Nepal. This report describes the results of the first two steps whose main purpose was to confirm that the modified reagent works well. For these two steps, test strips were made by dipping the filter strip end in the reagent solution, and left to dry. The test was performed by dipping the prepared filter strip in a bottle of urine. In Step 1, samples of urine known to contain protein (n=289) were compared to the new test. In Step 2, the urine samples (n=630) from antenatal clients attending the maternity hospital in Kathmandu were tested using dipstick, new test and Esbach test to determine sensitivity and specificity.

The specificity obtained was 99.2%, and sensitivity was 62.5%. We also determined that there was considerable inter-observer differences in interpreting gradations of color change in the dipstick test, a problem not encountered with the dichotomous new test. We found lower than expected rate of proteinuria overall. The study has further informed the choice of the threshold of the dichotomous color change, and the reagent will be modified to improve sensitivity in the follow on steps. In Steps 3 and 4, new test strips will be made at point of use from the pen platform, and tests will be done by voiding urine directly onto the prepared test strip.

This is a preliminary report for the first two parts of the study; a full report will be made available once all four steps are completed.

# 1. Introduction

## PURPOSE

This report summarizes the Step 1 and 2 of a study "New Protein Self-Test for Early Detection of Pre-Eclampsia". Specifically, these two steps describe how a reengineered reagent for detecting protein in urine performs against currently used tests. The validation was carried out at Paropakar Maternity and Women's Hospital (PMWH) in Kathmandu, Nepal in August 2010.

## BACKGROUND

Pre-eclampsia (PE) is a pregnancy complication accompanied by raised blood pressure, proteinuria and sometimes edema. Detection of pre-eclampsia is primarily based on the presence of hypertension and protein in the urine. Eclampsia (E) is defined as the development of convulsions or coma in a woman with pre-eclampsia. Untreated PE can lead to convulsions (eclampsia), multi-organ failure, Disseminated Intravascular Coagulopathy (DIC), and perinatal and maternal death. The condition also adversely affects the placenta and can result in poor intrauterine growth and premature birth or death of the fetus. Testing for increased proteinuria therefore is an important component for early diagnosis of pre-eclampsia.

Pre-eclampsia is more common in first-time pregnancies, and typically present with minimal or no warning. Early detection of pre-eclampsia and suitable treatment will significantly prevent mortality associated with this condition. PE/E is one of the leading causes of maternal deaths in the world—contributing between 8–25% of maternal mortality worldwide[1]. The Nepal Maternal Mortality and Morbidity Study (NMMMS) conducted in eight of 75 districts by the Family Health Division (FHD) of the Ministry of Health and Population (MoHP) in Nepal in 2008–2009 shows that Preeclampsia/eclampsia is the cause of 21% of maternal deaths in Nepal, now the second leading cause of maternal deaths after PPH, which accounts for 24% of maternal deaths[2].

The risk of women dying in pregnancy or childbirth continues at unacceptably high rates in developing countries, particularly in sub-Saharan Africa and Southern Asia. In order to achieve the Millennium Development Goal (MDG) 5, the global community must innovate and support substantial improvements in obstetric care. PE/E are among the top causes of maternal mortality and morbidity worldwide, ranking second only to hemorrhage as a specific direct cause of maternal mortality and disproportionately affecting women in developing countries[3,4]. Despite the prognostic importance of proteinuria, it remains a poorly utilized clinical test in pregnancy in developing nations. Widespread use of current clinic or bedside tests are limited by cost, time, training needs, laboratory infrastructure and the

[1] Khan K, et al. WHO analysis of causes of maternal death: a systematic review. Lancet 2006; 367: 1066–74
[2] Family Health Division, Options UK, New Era and CREHPA. 2009. Nepal Maternal Mortality and Morbidity Study 2008/2009. Nepal
[3] Khalid S Khan, Daniel Wojdyla, Lale Say, A Metin Gülmezoglu, Paul F A Van Look. "WHO analysis of causes of maternal death: a systematic review." Lancet 2006; 367: 1066–74
[4] World Health Organization (WHO). 1994. Mother-baby package: Implementing safe motherhood in countries. Geneva.

need for multiple visits to the facility by women where the tests can be reliably performed[5]. These are significant constraints in countries where few women receive quality focused antenatal care.

Worldwide, there is a significant unmet need for protein testing. For example, data from the Demographic and Health surveys (DHS) in Table 1 show that between 38-81% of pregnant women did not get proteinuria test during their last pregnancy. In Nepal 30% of pregnant women attend ANC fourth visit, but most do not get the protein test due to cost or unavailability[6]. A simple inexpensive protein test would increase detection of pre-eclampsia, especially if it were possible to do this at home in the community. This study conducted at PMWH helped to refine and validate a low-resource appropriate, color-based test for detection of proteinuria, and a key indicator of pre-eclampsia. Once fully evaluated, this simple inexpensive test can be used in the community and at the household level to support Nepal's national effort to achieve the MDG 5 to reduce the maternal mortality ratio, as PE/E accounts for 21% of all maternal deaths in Nepal[7].

**Table 1: Unmet need for early detection of Pre-eclampsia**

## Massive unmet need for early detection of PE Source DHS

| Country | % Unmet need for BP Check | % Unmet need for Proteinuria Check |
|---|---|---|
| Bangladesh | 53.1% | 70.5% |
| Bolivia | 24.5% | 50.9% |
| DRC | 38.8% | 57.8% |
| India | 52.5% | 56.8% |
| Indonesia | 13.9% | 63.0% |
| Kenya | 22.8% | 38.9% |
| Malawi | 28.6% | 81.3% |
| Mozambique | 48.7% | 73.9% |
| Nepal | 43.8% | 77.7% |
| Zimbabwe | 14.0% | 39.8% |

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[5] Maybury, H. and Jason W. "Proteinuria in Pregnancy: Just what is Significant?" Fetal and Maternal Medicine Review, 2004. 16:1 71-95

[6] Nepal Demographic and Health Survey 2006.

[7] Family Health Division, Options UK, New Era and CREHPA. 2009. Nepal Maternal Mortality and Morbidity Study 2008/2009. Nepal

## SCREENING METHODS OF PE/E: URINE PROTEIN TESTING DURING PREGNANCY

Some conventional tests for proteinuria are Dipstick Test, Boiling Test, Heller's Nitric Acid Test, John's Picric Acid Test, Ferrocyanid of Potassium and Acetic Acid test, Magnesium Nitric Test and Esbach test. We only describe those tests that are relatively inexpensive and do not require sophisticated biochemistry equipment.

***Dipstick Test*:** This is the leading method for proteinuria screening. The dipstick test is designed for lab or clinic, strips are small and to be used when urine is collected in a bottle. The reagent is impregnated into a cellulose strip pad on a plastic strip. The test result is semi-quantitative based on a gradual color change from pale green/ yellow to dark green/ blue. These dipsticks generally retail for approximately $40–50 per pack of 100, or 40 to 50 cents per strip. Off-brand dipsticks can be sold for as low as $10/100 (10 cents per strip); however, these dipsticks may not be as effective as name-brands[8,9]. The source of cost data are government medical stores and commercial outlets in Nepal and India. These costs do not include the cost of the clean/sterile bottle that is usually needed for a dipstick test. They usually have a shelf life extending into years, although this depends on stable temperatures and humidity. Once packages are opened (which usually include about 100 dipsticks), they should be used within a few weeks before they deteriorate.

***Boiling Test***—this is a common, easy and relatively reliable test for urine albumin. After adding one or two drops of acetic or nitric acid in one-third full of urine in test tube and boiling, the upper portion becomes turbid if there is presence of albumin. Then add a few drops of nitric acid, which will thicken the turbidity if albumin is present in the urine, and clear it if it is absent.

***Heller's Nitric-Acid Test***—this is not a reliable test. Once we put a little nitric acid into the test-tube and then putting little urine down the side of the test tube, a white ring is formed at the point of contact. From this white ring (thickness/viscosity), we interpret the amount of albumin present in the urine.

***Johnson's Picric-Acid Test***—when we put the few drops of picric acid on the top little urine in a test-tube, a turbidity or white zone immediately forms at the point of junction. If there is turbidity, heating of urine gives more clear result on presence of urine albumin.

***Ferrocyanid-of-Potassium and Acetic-Acid Test***—after adding few drops of potassium-ferrocyanid solution in a test-tube with one-third full of urine and thoroughly mixing the urine and the reagent, we need to add ten to fifteen drops of acetic acid on this. If albumin is present, a cloudiness more or less pronounced takes place, depending upon the amount of albumin present.

***Magnesium Nitric Test***—to five volumes of the saturated solution of sulphate of magnesium adds one volume of strong nitric acid. Fill a test-tube one-third full of this solution, and with a pipette allow the urine to flow down the side of the tube; at the point of contact a cloudy ring will form.

***Quantitative Test***—this test is used to determine the proportion of albumin per thousand. For this test, we use Esbach's albuminometer—bear two marks: one U, indicating the point to which the urine must be added; and one, R, the point to which the reagent is added. The

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[8] Khalid S Khan, Daniel Wojdyla, Lale Say, A Metin Gülmezoglu, Paul F A Van Look. "WHO analysis of causes of maternal death: a systematic review." Lancet 2006; 367: 1066–74

[9] World Health Organization (WHO). 1994. Mother-baby package: Implementing safe motherhood in countries. Geneva.

tube is filled to- U with filtered albuminous urine, and the reagent added till the point R is reached and closed with a stopper after inverting twelve times. It is then set aside for twenty-four hours. In the presence of albumin, the precipitate settles down. Based on this, we directly read off the precipitation in amount per mile, in grams.

Not all these tests are accurate and many require trained laboratory personnel, and are not well suited for point of care diagnosis. They are either dependent on laboratory infrastructure, designed for use by only trained health providers, or are expensive for most low-resource settings. The dipstick test (e.g., albustick) is very convenient, and easy to use in clinic settings, but the narrow strips were not designed for self-testing, and the cost of these tests is often a barrier for widespread use. The new protein test that we are testing will be of significantly lower cost, easy to interpret, and designed for self-test.

# 2. Development of the New Protein Self-Test

Jhpiego in collaboration with Johns Hopkins University School of Biomedical Engineering Center for Bioengineering Innovation and Design (JHU-CBID) has reengineered the protein test to be suited for use by semi and non-literate users, a test that yields a very sharp color change when significant proteinuria is present. Traditional dipstick tests have a range of 5 subtle color changes according to level of protein in urine. In addition, rather than manufactured test strips, the reagent is supplied in a felt tip marker pen, and test strip is made by marking the end of a strip of filter paper with the reagent. Once dry, the client can void urine directly on the strip, and a sharp color change will indicate significant proteinuria. This report describes the Steps taken in developing the reengineered reagent and the validation of the reagent, and not the reagent dispenser.

## MISSION AND DESIGN GOALS AND CONSTRAINTS

Our mission is to develop an alternative, low-cost proteinuria screening system to help screen for preeclampsia in women who otherwise would not have access to this detection due to geographic or economic barriers. This proteinuria screening system features an alternative detection mechanism that is cheaper than the current technology while simultaneously not sacrificing accuracy and consistency of results. This system will also not require any significant level of training or qualifications for proper and effective use.

### Design Goals

- Design a proteinuria screening system that uses an indicator on compatible test paper to determine the amount of protein in urine
- Design the device to display easy-to-read results without further analysis
- Design the device to withstand a max temperature of 50°C, while also having a working temperature range of 0°C to 40°C
- Design the device to withstand stress tests of various types such that the device remains functional during field use
- Develop the device such that it can be used by people of varying national, cultural and language backgrounds

### Design Constraints

- The final design should be cost-effective for developing nations; i.e. 30 tests for under $0.10.
- It should have a shelf life of at least 2 years.
- It should be versatile such that it can be used for other chemical tests with only minimal modifications.
- It should be small (<100 $cm^3$) and low in weight (<12oz) such that it can be carried by an individual for several hours without becoming a burden.
- It should be easy to use such that no prior training or qualifications are necessary for effective use.
- It should have a low incidence of false negatives (< 1%).

## PROTOTYPE DESIGN

The prototype design is a cheap, convenient, and effective proteinuria test. The test utilizes a reagent solution containing tetrabromophenol blue, which indicates the presence of protein in the urine. The reagent solution can be applied to filter paper at the testing site using a marker. The patient using the test will urinate on the test, and the resulting color of the test will indicate the presence or absence of protein in the urine. Ultimately, the design described below meets our price goal, and preliminary testing indicates that it has a high level of sensitivity and specificity.

### Marker

The main component of the screening kit is a marker that dispenses a protein-detecting solution. The marker, which is 6½" long and ½" in diameter, is easy to handle and has a convenient lanyard attached to the cap. It is quite sturdy because it is made of polycarbonate, which is impact resistant. The body of the marker is yellow, which is the color of the protein-indication solution when it is not exposed to protein above a certain threshold. The cap of the marker is blue, which is the color of the solution when it is exposed to protein above a threshold concentration. Reagent is dispensed through the wool felt nib. In addition to the marker, the kit contains the filter paper. Tests are made by using a marker to apply a protein-indicating solution to Whatman grade 1 filter paper. Other acceptable types of filter paper include Whatman grades 3, 3M, 4, and 42; Schleicher and Schuell grade 604; and VWR grade 610. However, these types of paper have not been used in tests yet.

To prepare the test, a local volunteer can use the marker to apply protein-indicating solution to filter paper. If the solution appears yellow on the filter paper, the volunteer can give the test strip to a pregnant woman. The volunteer then instruct the pregnant woman to void urine on the test for a fraction of a second midstream and report the resulting color of the test. To ensure that the filter paper strip remains stiff and allow voiding directly on it, the client is shown how to fold the non-reagent end lengthways so that for increased sturdiness. If the test remains yellow following urination, she knows she does not have proteinuria. If the test turns greenish-blue, she will be instructed to seek further medical attention.

### Reagent Solution

The reagent solution consists of several components that work together to produce an optimum color change in the presence of protein. The main color-changing indicator is Tetrabromophenol Blue. This compound is an acid-base indicator that appears yellow in solutions with pH of around 2.5-3 and below and appears greenish blue in solutions with a pH around 4-4.5 and above. However, it has been shown that this indicator will also turn greenish-blue in the presence of protein, even if the pH of the environment is 2.5 or below. Therefore, our solution contains an acid buffer composed of Citric Acid and Sodium Citrate. Our solution uses this acid buffer to maintain the pH of the solution around 3, so that a spot of our solution appears yellow on paper or cotton. Then when urine comes into contact with the solution on paper, the Tetrabromophenol Blue causes the color to change from yellow to greenish blue if the urine has a sufficient amount of protein, indicating a positive test. If there is not enough protein in the urine, the spot will remain yellow. Tetrabromophenol Blue does not easily dissolve in water, but it does in certain alcohols. Because of this, the solvent of the solution contains water and a small amount of Isopropyl Alcohol (about a 3:1 ratio of water to alcohol).

The following is a list of materials and supplies needed in order to reproduce our prototype. Please note that the amounts of materials specified are conservative, meaning that not all of every material is used.

Solutions: 1 L Water, 1 L 70% Isopropyl Alcohol, 100 g Citric Acid, 100 g Sodium Citrate, 1 g Tetrabromophenol Blue,100' roll Filter Paper: Whatman Grade 1

## Preliminary Testing of Reagent

The preliminary testing of the reagent was conducted at the JHU CBID laboratory in three tests as described below. Samples of frozen, anonymous urine from the Pathology Lab in the Johns Hopkins University Hospital was thawed and used in Test 1, 2& 3:

### *Test 1: Using Filter paper*

**Methods:**

- Thawed the following urine solution with varying protein concentrations (mg/dL):
  - Sample number 2 (5.3 mg/dl)
  - Sample number 81 (11.7mg/dl)
  - Sample number 69 (46.4mg/dl)
  - Sample number 64 (86.7mg/dl)
- Prepared 8 filter paper tests using "Iso 4–1" Solution.
- Prepared 4 more filter paper tests with "Iso 4–1" reagent that was made in August 2009. (Stored in plastic bag for previous 6 months).
- Added 2mL of each urine sample to 2 filter paper tests.
- Added 1mL urine to 6 month old tests (which were cut in half). A 5mL syringe with needle was used to apply the urine.

**Results:**

The test remained yellow with the 5.3 and 11.7 mg/dL solutions (the 11.7 began to show a slight hint of green). With 46.4 mg/dL, the test was mostly light green. The 86.7 mg/dL solution turned the test mostly green with some blue beginning to show. The 6-month old tests (the half circles) seemed to show consistent results with the tests that were made only a few moments before. This is a good sign that our test can be stored for significant lengths of time.

### *Test 2: Using Nepali handmade paper (frozen urine sample)*

**Method:**

- Thawed the following urine solutions with varying protein concentrations (mg/dL):
  - Sample number 2 (5.3 mg/dl)
  - Sample number 81 (11.7mg/dl)
  - Sample number 69 (46.4mg/dl)
  - Sample number 64 (86.7mg/dl)
- Cut four strips of the 40gm Nepali paper. Used a dropper to add the "Iso 4-1" solution until half of the strip was covered. Waited for the solution to dry.
- Cut 3 strips of 20, 40, and 60 gm Nepali natural paper.
- Dipped strips into "Iso 4–1" solution and waited for paper to dry (about 5 min)
  - * *Green solution dried yellow on paper*

- Took one of each strip (20/40/60) and placed under running Distilled water.
    * *Yellow mark on paper remained yellow (this was a good sign; most other paper turned blue when placed in running water)*
- Added 2mL of artificial urine with protein concentrations of 40 and 150 mg/dL to the remaining two strips of each type.

**Results:**

In all three cases, the 40mg/dL test began to turn green and the 150mg/dL was completely green. The tests on the 20gm paper appeared somewhat translucent. We could not tell any significant difference between the 40 and 60gm paper. Recommend using the 40gm paper.

## *Test 3: Using Nepali handmade paper (Real Urine)*

The tests seemed to be working on the Nepali paper, so we decided to run a test using the real urine samples.

**Method:**

- Used syringe to squirt 2mL of each urine solution to each test strip:
    - 5.3 mg/dl
    - 11.7mg/dl
    - 46.4mg/dl
    - 86.7mg/dl

**Results:**

The 5.3mg/dL test remained mostly yellow with a slight bit of green showing. Both the 11.7 and 46.4mg/dL were about half yellow/half green (the 46.4 showed a little greener than the 11.7). The 86.7mg/dL test turned a clear blue color.

Overall, the conclusions from preliminary testing of the reagent are that it is feasible for our device to work on something other than the filter paper we have been using. In our opinion, the filter paper shows a much smoother transition in colors, however, if we only want to detect concentrations of 70–80mg/dL and above, then this natural paper should be fine.

# 3. New Protein self-test Validation Tests in Nepal

## OBJECTIVES

As part of the validation for the new protein self-test, the 2 steps being reported here are:

**Step 1:** Perform the new test on known protein positive specimens of urine and confirm results using standard dipstick urinalysis and the Esbach test in a laboratory setting.

**Step 2:** perform the new test on all antenatal clients attending PMWH, Kathmandu and compare to standard dipstick, and Esbach tests to determine sensitivity, specificity, and predictive value of the new protein test in a laboratory setting

In addition, subsequent steps planned are:

**Step 3:** Determine the acceptability of self-testing for proteinuria by Nepali ANC clients (in clinic setting). This component of the study will measure specifically.

a. Women's ease and willingness to use the self-test
b. Women's ability to recognize the colour change as compared to that of the trained observer

**Step 4:** To assess the acceptability and feasibility of utilizing this Proteinuria Self-Test at the community level (Step 4)

Step 1 and Step 2 were completed, and this is a preliminary report that documents the findings from Step 1 and Step 2 only. A comprehensive report for the entire study will be prepared after completion of all four steps.

## METHODOLOGY

The research protocol was approved by the Johns Hopkins School of Public Health International Review Board (IRB) as well as the Nepal Health Research Council (NHRC). For the Steps 1 and 2 of the study, the reengineered reagent was set to change color at 70mg/ dl of protein because at this level the color change was the sharpest and the level represents the approximate midpoint between 30 mg/dl (1+) and 100mg/dl (2+) on a standard dipstick generally thought to be level of pathological and actionable proteinuria. The test strips were prepared by dipping the tip of a filter paper strip 1 cm wide by 8cm long into the reengineered reagent and left to dry. The test was performed by dipping the strip into a bottle of urine, and observing for color change. If there was a color change from yellow to greenish blue, test was regarded as positive.

Specimens were obtained from the laboratory after the lab had performed its own test. For Step 1 we collected all specimens that were identified to be positive for any amount of protein. Urine samples included those from pregnant and non-pregnant patients. For Step 2, we collected all the specimens of urine from the laboratory that came from antenatal patients only. All patient identifiers were removed from these samples before reaching the study team. No identifiers were collected on clients.

Once received by the study team, a standard dipstick test was performed on all samples by a study nurse, and another study team member performed the new test on all specimens and set up the Esbach test on specimens that had sufficient urine (10 mls).

### Step 1

All urine specimens received at the PMWH laboratory are routinely tested for protein by hospital staff using dipstick. 289 urine specimens identified to be positive for any amount of protein on standard dipstick testing were collected from ANC outpatient Department (OPD), emergency laboratory, and parasitology department and wards from PMWH. The routine testing is generally done by nurses, patient attendants or laboratory personnel. After stripping specimen bottles of patient identifier information, all samples were subjected to a repeat dipstick test by a trained nurse, and then subjected to the new test performed by one of the two researchers, who also set the Esbach test, which was read the next day after 24 hours.

### Step 2

All clients attending ANC at the PMWH undergo protein testing as a matter of routine. The routine testing is generally done by nurses, or by patient attendants, not laboratory personnel. We included in the study, urine specimens from the first 50 pregnant women seen daily. The specimens were collected in a bottle, first tested by clinic personnel using the standard dipstick. All the specimens than were tested by the study nurse using dipstick, and then tested by one of 2 researchers using the new test and the Esbach test. A total of 630 samples were examined.

## INTEROBSERVER ERROR

In order to test the inter observer error or the internal consistency of results for the dipstick and the protein self-test, one known specimen of proteinuria where large quantity of urine was available was divided into 20 portions and ran as different specimens through the entire system including the clinical provider as well as the research provider.

## RESULTS

**Step 1:** How well does the new test pick up all significant proteinuria:

**Table 2: Results of Standard Dipstick Test performed by Study Nurse on all Specimens Identified as Positive for Proteinuria by PMWH**

| Proteinuria Level | Number | % |
|---|---|---|
| Nil | 46 | 15.9 |
| Trace | 148 | 51.4 |
| 1+ (0.3g/l) | 47 | 16.3 |
| 2+ (1g/L) | 33 | 11.5 |
| 3+ (3g/L) | 12 | 4.2 |
| 4+ (10g/l) | 2 | 0.7 |
| **Total** | **288** | **100** |

*(1 specimen result not recorded)*

**Comments:**

15.9% of the specimens received from PMWH and identified to have some proteinuria were not found to have any protein when tested by our trained study nurse. Specimens are tested by many clinical as well as non-clinical staff at this hospital. Occasionally these tests are done by patient attendants who have been trained on the job to do so.

**Table 3: Results of New test on all specimens identified to be positive for proteinuria by PMWH**

| Significant Proteinuria | Number | % |
|---|---|---|
| Negative | 241 | 83.4 |
| Positive | 48 | 16.6 |
| **Total** | **289** | **100%** |

**Table 4: Correlation between Dipstick Test Result and New Test**

| Dipstick Test | New Test | |
|---|---|---|
| | Negative | Positive |
| Nil | 46 | 1 |
| Trace | 146 | 2 |
| 1+ (0.3g/l) and above | 49 | 45 |

**Comment:**

Of the 49 tests that were 0.3g/l (1+) and above on dipstick, the new test picked up 45 (92%)

**Table 5: Results of Esbach Test on all Specimens Identified to be Positive for Proteinuria by PMWH (n=193)**

| Protein level g/l | number | % |
|---|---|---|
| 0 g/l | 9 | 4.7 |
| 0.05-025 g/l | 108 | 56.0 |
| 0.30-0.65 g/l | 50 | 25.9 |
| 0.7-0.95 g/l | 4 | 2.1 |
| 01.0-3.0 g/l | 20 | 10.4 |
| 3+ g/l | 2 | 1.0 |
| **Total** | **193** | **100%** |

**Table 6: correlation between Esbach test and new test (n=193 specimens)**

| | New test | |
|---|---|---|
| **Protein level g/l** | **Negative** | **Positive** |
| 0 g/l | 9 | 0 |
| 0.05-025 g/l | 108 | 0 |
| 0.30 and up g/l | 42 | 34 |

**Comment:**

Of the 42 specimens that had significant proteinuria on Esbach (0.30g/l and above) 34 (81%) were identified by the new test (81%). 7 Esbach tests were not set as volume of urine was insufficient. All results for these specimens were excluded from this analysis.

**Step 2:** What are the test characteristics of new protein test.

- The prevalence of proteinuria in antenatal population confirmed by regular dipstick screening (performed by one trained study nurse) and Esbach test was found to be 1.2% at 1g/l threshold and 5.6% at 0.3g/l.

**Comment:**

The prevalence of proteinuria is lower than anticipated. Possible reasons could be that there is a real lower than expected prevalence of proteinuria, or that patients who do have PEE are systematically excluded from ANC as they get admitted and managed.

- The test characteristics of the new test done on 630 specimens as compared to Standard dipstick test as performed by study nurse and result verified by Esbach confirmation are as follows:
  - Sensitivity: 62.5%
  - Specificity 99.2%
  - Positive predictive value: 88%
  - Negative predictive value: 99.5%

**Comments:**

The sensitivity was somewhat lower than reported for standard dipstick (about 75%) Other characteristics are comparable. One way to increase sensitivity would be to lower threshold for positivity.

## OTHER FINDINGS:

20 aliquots were made from urine collected from one patient and submitted for testing first by the hospital system ( nurses, and other staff in ANC), and then study with dipstick, new test and Esbach.

a. Hospital staff read the standard dipstick results as trace (4), 1+(7), 2+ (8) and 3+(1). Study nurse read standard dipstick as 1+(2) and 2+(18)
b. All 20 specimens were read as positive using the New test
c. Esbach results ranged as 0.9g/l (7), 1g/l 9 (13)

### Comment:

Hospital staff (several nurses and ANC staff had a significant inter-observer differences. Based on Esbach results, all the standard dipstick results should have been read as 2+. While the new test was not subjected to different observers, we found no intra-observer difference.

# 4. Discussion

All three tests used in this study are subjective to some extent. The standard dipstick test probably has a greater level of inter as well as intra observer differences as color changes between trace, 1+, 2+ are fairly subtle. The new test has a lower intra observer difference as color changes are sharp. The high interobserver error observed for dipstick suggests that many clinic providers do not read slight changes in color very well whereas the color change in the new protein self-test which is dichotomous is easily readable. The reading of Esbach is also prone to some minor level of subjectivity.

The data suggest reasonable sensitivity and with very high specificity. One way to improve the sensitivity of the new test would be to lower the threshold of the test from 0.7g/l to 0.3 g/l, The improved sensitivity will catch all significant proteinuria, but would perhaps decrease specificity somewhat, with some false positives. This modification requires adjusting the ratios of the chemicals we are currently using in the reagent. WE already know from preliminary studies that we can get a reasonably good color change at 0.3g/l level, but this will not be as sharp as the change we have now. Additional modifications will be needed to get the same enhanced color change.

**Recommendations for next phase of study:**

1. Further reengineer reagent to obtain sharp color change at 0.3g/l.
2. Ensure urine is collected as soon as client reaches clinic as it is common for clients to void urine on arrival from afar before collection bottle has been provided. Also, the narrow mouth bottles supplied by hospital must be replaced by wide mouth bottles to ensure sufficient and convenient collection.
3. In Step 3, the full platform (reagent in pen, test strip prepared on site) as well as urine voided directly on strip will be tested.

**Important Note:** This report is a preliminary report for a 4 part study. 2 parts remain to be completed. The results herein are only meant for reporting purposes to donor. Distribution of these results beyond this purpose may jeopardize chances of publication in peer reviewed journals which is planned for after the full study is completed.

# Acronyms and Abbreviations

| | |
|---|---|
| ANC | Antenatal Care |
| BP | Blood pressure |
| DHS | Demographic and Health Survey |
| DIC | Disseminated Intravascular Coagulopathy |
| E | Eclampsia |
| FHD | Family Health Division |
| GoN | Government of Nepal |
| IRB | International Review Board |
| JHU-CBID | Johns Hopkins University School of Biomedical Engineering Center for Bioengineering Innovation and Design |
| MDG | Millennium Development Goal |
| MCHIP | Maternal and Child Health Integrated Program |
| MoHP | Ministry of Health and Population |
| NHRC | Nepal Health Research Council |
| NMMMS | Nepal Maternal Morbidity and Mortality Study |
| PE/E | Pre-eclampsia/eclampsia |
| PPH | Postpartum hemorrhage |
| PMWH | Paropakar Maternity and Women's Hospital |
| R | Reagent |
| U | Urine |